Anal. Calcd. for $C_{17}H_{17}NO_4$: C, 68.25; H, 5.75; N, 4.69. Found: C, 68.34; H, 5.80; N, 4.69.

(−)-2-(N-Carbobenzoxyanilino)propionpiperidide (XII).—A cooled solution of 4 g. (0.013 mole) of X in 10 ml. of methanol was titrated with 1 *M* methanolic NaOH or NaOMe to a phenolphthalein end point. The methanol was removed and replaced with a mixture of ether-Skellysolve B. After the material was collected and dried at 65° (0.1 mm.) for 36 hr., there was obtained 3.16 g. of sodium salt. This salt, partially soluble in ether, was placed in 20 ml. of ether and cooled to about 0°. To the stirred mixture was added dropwise, 1.4 g. (0.011 mole) of oxalyl chloride in 10 ml. of ether. After all the gas had evolved (15 min.), 1.87 g. (0.02 mole) of piperidine was added to the cooled, stirred mixture over a 15-min. period. The mixture was allowed to stand for 0.5 hr. and filtered. The ether solution was washed with 1 *M* HCl, then with 1 *M* $NaHCO_3$, dried over Drierite, and the ether was removed *in vacuo*. There was obtained 2.41 g. of an oil which was chromatographed on a silicic acid-chloroform column. The first fraction (0.42 g.) consisted mainly of XVI, an oil; λ_{max} 5.40, 5.60 (anhydride), and 5.86 μ (carbamate). The second fraction (1.54 g.), which contained the desired product, was recrystallized several times from ethyl acetate–Skellysolve B to afford 0.50 g. of XII, m.p. 74–76°, $[\alpha]^{23}D$ −60° (2% in ethanol); λ_{max} 5.88 (carbamate) and 6.03 μ (amide).

Anal. Calcd. for $C_{22}H_{26}N_2O_3$: C, 72.21; H, 7.15; N, 7.65. Found: C, 71.96; H, 7.07; N, 7.32.

The infrared spectrum of the above compound was identical with that of racemic XII, m.p. 93–95°, prepared by treating 2.32 g. (0.01 mole) of racemic XIII with 2 g. (0.022 mole) of benzyl chlorformate in a mixture of 15 ml. each of 1.5 *M* $NaHCO_3$ and $CHCl_3$. The mixture was stirred vigorously for 6 hr. and the chloroform layer was separated from the aqueous phase. After washing the organic phase with 1 *M* HCl, it was dried over Drierite and the solvent was removed. The oil (2.24 g.) solidified on standing and was recrystallized from ethyl acetate-Skellysolve B.

(−)-2-Anilinopropionpiperidide (XIII).—Intermediate XII (0.47 g., 0.001 mole) in 10 ml. of methanol was shaken for 17 min. with 0.1 g. of palladium on carbon under hydrogen at 1.76 kg./cm.^{2} (25 p.s.i.). The mixture was filtered and the catalyst was washed with methanol. The solvent was removed *in vacuo* to afford 0.26 g. of XIII, m.p. 63–65°, $[\alpha]^{23}D$ −13° (1% in ethanol). The infrared spectrum of XIII was identical with that of the racemic compound.[1]

(−)-N^1-Pentamethylene-N^2-phenyl-1,2-propanediamine Dipicrate.—To a stirred mixture of 0.082 g. (0.002 mole) of $LiAlH_4$ in 2 ml. of tetrahydrofuran was dropped 0.25 g. (0.001 mole) of XIII in 2 ml. of tetrahydrofuran. The mixture was refluxed for 5 hr., then treated successively with 0.1 ml. of water, 0.2 ml. of 15% NaOH, and 0.1 ml. of water, and filtered. The solvent was removed *in vacuo* and the residue was dissolved in 1 ml. of ethanol. Enough saturated ethanolic picric acid was added to produce complete precipitation. The yield of XIV dipicrate, m.p. 132.5–134.5°, was 0.56 g. Recrystallization from ethanol afforded 0.40 g. of the salt, m.p. 134–135.5°, $[\alpha]^{23}D$ −60° (2% in acetone).

Anal. Calcd. for $C_{36}H_{38}N_8O_{14}$: C, 46.25; H, 4.23; N, 16.61. Found: C, 46.31; H, 4.41; N, 16.51.

Hydrolysis of (−)-Phenampromide (IV).—A mixture of 3 ml. of concentrated H_2SO_4, 3 ml. of water, and 1.37 g. (0.005 mole) of (−)-phenampromide[3] was heated on a steam bath for 15 hr. The solution was made basic with 4 *N* NaOH and extracted with ether. The organic phase was dried, and the solvent was removed *in vacuo*. To the residue was added 4 ml. of ethanol and enough saturated alcoholic picric acid to ensure complete precipitation. The yield of XIV dipicrate, m.p. 133.5–135.5°, was 1.74 g. Recrystallization from ethanol afforded 1.48 g., m.p. 134–136°, $[\alpha]^{23}D$ −53° (2% in acetone), of XIV dipicrate. No melting point depression was observed when the salt was mixed with the dipicrate of the diamine XIV obtained from IXa. The infrared ($CHCl_3$) spectra of the dipicrates obtained by different routes were identical.

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Substituent Constants for Aliphatic Functions Obtained from Partition Coefficients

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From the partition coefficients between 1-octanol and water of a variety of derivatives of the type $C_6H_5(CH_2)_nX$, the partition constants (π) for the aliphatic functions X have been determined. The practical value of the additive character of π for the correlation of biological activity with chemical structure is illustrated with data on the narcotic action of alcohols, esters, ketones, and ether on tadpoles. The relation of π to ΔR_M (a chromatographically determined substituent constant) is shown.

Recently we have shown that substituent constants can be useful in the quantitative correlation of biological activity with chemical structure.[3] In particular, we have found that using electronic parameters such as the Hammett σ-constant, pK_a values, or electron densities obtained from molecular orbital calculations with a substituent constant π ($\pi = \log P_X - \log P_H$) obtained from partition coefficients, mathematical expressions could be found for correlation in a wide variety of structure–activity problems. π is a free-energy-related constant for a functional group and is similar to σ. For example, π for the CH_3 group is found by subtracting the logarithm of the partition coefficient for benzene (P_H) from that of toluene (P_X). We have used 1-octanol–water for the solvent system. In evaluating π from partition coefficients obtained with over 200 aromatic compounds, we have observed that π for a given function remains approximately constant in much the same fashion as does σ as long as no strong group interactions occur.[4]

We now report values for functional groups not attached to an aromatic nucleus. These too appear to be approximately constant when strong group interactions are absent.

Table I gives the logarithm of the partition coefficients for a variety of compounds of the type $C_6H_5(CH_2)_nX$. The phenyl group was included for analytical con-

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(3) (a) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964); (b) C. Hansch and A. R. Steward, *J. Med. Chem.*, **7**, 691 (1964); (c) C. Hansch, A. R. Steward, and J. Iwasa, unpublished results.

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TABLE I

LOGARITHM OF THE OCTANOL–WATER PARTITION COEFFICIENTS

X	Log P $C_6H_5(CH_2)_3X$	$C_6H_5(CH_2)_2X$	$C_6H_5CH_2X$	C_6H_5X
H	3.68 ± 0.01[a]	3.15 ± 0.01[a]	2.69 ± 0.01[a]	2.13 ± 0.01[a]
F	2.95 ± 0.02			2.27 ± 0.01
Cl	3.55 ± 0.02	2.95 ± 0.01		2.84 ± 0.02
Br	3.72 ± 0.01	3.09 ± 0.02		2.99 ± 0.01
OH	1.88 ± 0.01	1.36 ± 0.01	1.10 ± 0.02	1.46 ± 0.01
NH_2	1.83 ± 0.02	1.41 ± 0.01	1.09 ± 0.02	0.90 ± 0.01
$COOCH_3$	2.77 ± 0.01	2.32 ± 0.01	1.83 ± 0.02	2.12 ± 0.02
COOH[b]	2.42 ± 0.01	1.84 ± 0.01	1.41 ± 0.01	1.85 ± 0.01
CN	2.21 ± 0.01	1.72 ± 0.02	1.56 ± 0.02	1.56 ± 0.01
$COCH_3$	2.42 ± 0.01		1.44 ± 0.03	1.58 ± 0.01
$CONH_2$	1.41 ± 0.01	0.91 ± 0.01	0.45 ± 0.03	0.64 ± 0.01
$CH(^+NH_3)COO^-$	−0.36 ± 0.02			
$OCOCH_3$	2.77 ± 0.02	2.30 ± 0.02	1.96 ± 0.01	1.49 ± 0.01
OCH_3	2.70 ± 0.01			2.11 ± 0.01
$N(CH_3)_2$	2.73 ± 0.01			2.31 ± 0.01
$N(CH_3)_3{}^+I^-$	−2.02[c]			

[a] Standard deviation. [b] The values for the COOH which varies with the concentration were estimated as previously described.[4] [c] P for this substance varied considerably, depending on the concentration in the octanol phase. The value reported here is for a concentration of 2.62×10^{-5} M in the octanol phase. When this concentration increased to 20.6×10^{-5} M, log P was −1.57.

TABLE II

VALUES OF π FOR ALIPHATIC FUNCTIONS

X	log $P_{C_6H_5(CH_2)_3X}$ − log $P_{C_6H_5CH_2CH_2CH_3}$	log $P_{C_6H_5(CH_2)_2X}$ − log $P_{C_6H_5CH_2CH_3}$	log $P_{C_6H_5CH_2X}$ − log $P_{C_6H_5CH_3}$	log $P_{C_6H_5X}$ − log $P_{C_6H_5}$
F	−0.73 ± 0.03			0.14 ± 0.02
Cl	−0.13 ± 0.03	−0.20 ± 0.02		0.71 ± 0.03
Br	0.04 ± 0.02	−0.06 ± 0.03		0.86 ± 0.02
OH	−1.80 ± 0.02	−1.81 ± 0.02	−1.59 ± 0.03	−0.67 ± 0.02
NH_2	−1.85 ± 0.03	−1.74 ± 0.02	−1.60 ± 0.03	−1.23 ± 0.02
$COOCH_3$	−0.91 ± 0.02	−0.83 ± 0.01	−0.86 ± 0.03	−0.01 ± 0.03
COOH[a]	−1.26 ± 0.02	−1.31 ± 0.02	−1.28 ± 0.02	−0.28 ± 0.02
CN	−1.47 ± 0.02	−1.43 ± 0.03	−1.13 ± 0.03	−0.57 ± 0.02
$COCH_3$	−1.26 ± 0.02		−1.25 ± 0.04	−0.55 ± 0.02
$CONH_2$	−2.28 ± 0.02	−2.24 ± 0.02	−2.24 ± 0.04	−1.49 ± 0.02
$CH(^+NH_3)COO^-$	−4.04 ± 0.03			
$OCOCH_3$	−0.91 ± 0.02	−0.85 ± 0.03	−0.73 ± 0.01	−0.64 ± 0.02
OCH_3	−0.98 ± 0.02			−0.02 ± 0.02
$N(CH_3)_2$	−0.95 ± 0.02			0.18 ± 0.02
$N(CH_3)_3{}^+I^-$	−5.70[a]			

[a] See Table I.

venience for the spectrophotometric determination of the concentrations of compounds in the partitioned phases. It has the advantage over other ultraviolet-absorbing groups such as the carbonyl in that it is rather inert chemically and thus does not interact strongly with the functional groups whose π-values were being determined. Table II gives the "aliphatic" π-values for 14 functional groups. Also included in Tables I and II for purposes of comparison are the corresponding "aromatic" π-values. As one would expect, it is clear from Table II that π for a particular function changes considerably when resonance between the function and the aromatic ring is broken by a CH_2 group. Insulation of the resonance effect permits greater localization of the electrons and, therefore, greater relative solubility in the more polar aqueous phase where the greater possibility for hydrogen bonding occurs. As the aromatic ring is further removed from the function, π appears to become constant. The values for π when n is equal to 2 and 3 are, in most cases, constant within the limits set by experimental error.

Since π appears to be an additive constant as long as strong group interactions are absent, the values in Tables I and II allow one to estimate many log P values. For example, neglecting a very small contribution from log P_H, the value for methyl alcohol can be obtained as follows.

$$\log P_{C_6H_5(CH_2)_3OH} - \log P_{C_6H_5CH_2CH_3} = \log P_{CH_3OH} = -1.27 \quad (1)$$

$$\log P_{C_6H_5(CH_2)_2OH} - \log P_{C_6H_5CH_3} = \log P_{CH_3OH} = -1.33 \quad (2)$$

$$\log P_{C_6H_5CH_2OH} - \log P_{C_6H_5} = \log P_{CH_3OH} = -1.03 \quad (3)$$

Comparison of eq. 1, 2, and 3 again indicates that if the functional groups are removed by 2 or more CH_2 groups, group interaction is small and approximately constant values for log P can be additively determined.

The value for ethyl methyl ketone can likewise be calculated (eq. 4). The experimentally determined value is 0.32.

$$\log P_{C_6H_5(CH_2)_3COCH_3} - \log P_{C_6H_5CH_3} = \log P_{C_2H_5COCH_3} = 0.27 \quad (4)$$

From this work and that previously reported, a good general purpose value for the methyl or methylene group is 0.52. Thus, adding 1.04 to 0.32 gives 1.36 as the calculated value for butyl methyl ketone. The experimental value is 1.38. Such additivity does not hold, however, when new group interactions not possible in the parts occur in a molecule. For example,

log P for $CH_3COCH_2CH_2COOCH_3$ was found to be -0.23. Taking the value of 0.32 for ethyl methyl ketone and adding to this -0.91 for the $COOCH_3$ function gives a calculated value of -0.59. Apparently the polarizing effects of the two strongly electron-attracting functions interact to cause lower water solubility than simple addition would lead one to expect. Previous results[3a] indicate that electron withdrawal (in this case *via* an inductive effect) increases π for hydrogen-bonding functions, presumably by tighter binding of lone pair or π-electrons. This reduces the affinity of the function for the aqueous phase thus increasing log P or π. In the above keto ester it is possible that internal hydrogen bonding might also play a subsidiary role. As the groups are further separated, the interactions fall off and the calculated and experimental values come closer into agreement. The experimental value for $CH_3CO(CH_2)_4COOCH_3$ is 0.55. Using the value for butyl methyl ketone and -0.91 for $COOCH_3$ yields a calculated value of 0.47.

A good example of the practical side of the additive nature of log P for the correlation of chemical structure with biological activity comes from the work of Overton on the narcosis of tadpoles by alcohols, esters, ketones, and ether. The log $(1/C)$ values for the isonarcotic concentration are taken from the report of McGowan.[5] These and the calculated values of log P (Table III) were fitted to the equation log $(1/C) = k \log P + c$ by the method of least squares to produce eq. 5. For

$$\log (1/C) = 0.869 \log P + 1.242 \qquad n = 28 \quad r = 0.965 \quad s = 0.229 \qquad (5)$$

eq. 5, n is the number of points used in determining the constants, r is the coefficient of correlation, and s is the standard deviation. The correlation obtained with eq. 5 is quite good considering the problem of determining the isonarcotic concentration with tadpoles.

In calculating log P for a chain branching of the type $(CH_3)_2CH-$, -0.13 was subtracted from log P for the normal chain and for a tertiary grouping, *e.g.*, $(CH_3)_3C-$, and -0.22 was subtracted from log P for the straight chain. These values were obtained from log P for the corresponding phenoxyacetic acids.[4]

Correlations similar to that obtained by eq. 5 have been obtained using parachor[6] instead of log P. More recently, McGowan[7] has shown the relationship between π and parachor for a given function. Preliminary comparisons indicate both log P and parachor give similar results in evaluating the forces[8] which limit the concentrations of biologically active molecules reaching the sites of action. Log P or π would appear to be the more useful parameter, partly because of its ease of determination and partly because, through variation of the solvents, one should be able to develop a model system more closely approximating the biophases. In this connection, the work of Green and Marcinkiewicz[8] is of great importance. Using reversed-phase, tankless, flat-bed chromatography, they have shown that R_M, which was defined by Bate-Smith and Westall[9] as $R_M = \log [(1/R_F) - 1]$, is an additive and constitutive property just as log P is. They have introduced the term ΔR_M for effect of a substituent such as CH_3 upon the R_F value of a parent compound. As one would expect, there is indeed a very good linear correlation between π and ΔR_M as is revealed by eq. 6.

$$\pi = -1.103 \Delta R_M + 0.647 \qquad r = 0.970 \quad s = 0.051 \qquad (6)$$

Equation 6 was derived from the data in Table IV, using π-values[4] and ΔR_M constants[8b] obtained from phenols. In measuring R_M for the phenols, Trigol and diisopropyl ether were used as the two phases in the tankless chromatography. The excellent correlation obtained with eq. 6 is striking support for eq. 7 suggested by Collander.[10] Collander presented evidence

$$\log P_1 = a \log P_2 + b \qquad (7)$$

TABLE III

ISONARCOTIC CONCENTRATIONS OF ESTERS, ALCOHOLS, KETONES, AND ETHER WITH TADPOLES

Compd.	log P	−Log $(1/C)$− Obsd.	Calcd.	Δ log $(1/C)$
CH_3OH	−1.270	0.300	0.138	0.162
C_2H_5OH	−0.750	0.500	0.590	0.090
CH_3COCH_3	−0.730	0.650	0.607	0.043
$(CH_3)_2CHOH$	−0.360	0.900	0.929	0.029
$(CH_3)_3COH$	0.070	0.900	1.303	0.403
$CH_3CH_2CH_2OH$	−0.230	1.000	1.042	0.042
CH_3COOCH_3	−0.380	1.100	0.911	0.189
$C_2H_5COCH_3$	−0.270	1.100	1.059	0.041
$HCOOC_2H_5$	−0.380	1.200	0.911	0.289
$C_2H_5OC_2H_5$	0.590	1.200	1.754	0.554
$(CH_3)_2C(C_2H_5)OH$	0.590	1.200	1.754	0.554
$CH_3(CH_2)_3OH$	0.290	1.400	1.494	0.094
$(CH_3)_2CHCH_2OH$	0.160	1.400	1.381	0.019
$CH_3COOC_2H_5$	0.140	1.500	1.363	0.137
$C_2H_5COC_2H_5$	0.310	1.500	1.511	0.011
$CH_3(CH_2)_4OH$	0.810	1.600	1.946	0.346
$CH_3CH_2CH_2COCH_3$	0.310	1.700	1.511	0.189
$CH_3COOCH_2C_2H_5$	0.660	2.000	1.815	0.185
$C_2H_5COOC_2H_5$	0.660	2.000	1.815	0.185
$(CH_3)_2CHCOOC_2H_5$	1.050	2.200	2.154	0.046
$CH_3COOCH_2CH(CH_3)_2$	1.050	2.200	2.154	0.046
$CH_3COO(CH_2)_3CH_3$	1.180	2.300	2.267	0.033
$CH_3CH_2CH_2COOC_2H_5$	1.180	2.400	2.267	0.133
$CH_3(CH_2)_3COOC_2H_5$	1.700	2.700	2.719	0.019
$CH_3COO(CH_2)_4CH_3$	1.700	2.700	2.719	0.019
$C_6H_5COCH_3$	1.580	3.000	2.615	0.385
$CH_3(CH_2)_7OH$	2.370	3.400	3.301	0.099
$CH_3(CH_2)_3COO(CH_2)_3CH_3$	2.740	3.600	3.623	0.023

TABLE IV

CORRELATION OF π AND ΔR_M OF XC_6H_4OH

X	ΔR_M	π Obsd.	π Calcd.	π (obsd. − calcd.)
4-NH_2	1.762	−1.63	−1.297	0.333
3-NH_2	1.720	−1.29	−1.251	0.039
4-OH	1.450	−0.84	−0.954	0.114
3-OH	1.374	−0.66	−0.869	0.209
4-CN	0.488	0.14	0.108	0.032
3-CN	0.488	0.24	0.108	0.132
4-CH_3	−0.165	0.48	0.829	0.349
3-CH_3	−0.165	0.50	0.829	0.329
4-NO_2	0.281	0.50	0.337	0.163
3-NO_2	0.176	0.54	0.453	0.087
4-Cl	−0.165	0.93	0.829	0.101
3-Cl	−0.165	1.04	0.829	0.211

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TABLE V

LOG P VALUES FOR MISCELLANEOUS COMPOUNDS

Compd.	Log P
$C_6H_5C{\equiv}CH$	2.53 ± 0.01
$CH_3COC_2H_5$	0.32 ± 0.01
$CH_3CO(CH_2)_3CH_3$	1.38 ± 0.01
$CH_3CO(CH_2)_2CH{=}CH_2$	1.02 ± 0.01
$CH_3COCH_2CH_2$-◁	1.50 ± 0.01
Thiophene	1.81 ± 0.01
Indole	1.14 ± 0.01
Pyridine	0.65 ± 0.01
Quinoline	2.03 ± 0.01
$C_6H_5B(OH)_2$	1.58 ± 0.01
$CH_3COCH_2CH_2COOCH_3$	−0.23 ± 0.02
$CH_3CO(CH_2)_4COOCH_3$	0.55 ± 0.03
$CH_3(CH_2)_3SCN$	2.03 ± 0.02

that the logarithm of the partition of a compound in one set of solvents (P_1) is linearly related to the logarithm of the partition coefficient in a second similar set of solvents (P_2).

Since R_M and ΔR_M are so readily obtained *via* tankless chromatography, they should prove to be valuable supplements to log P and π in the extrathermodynamic substituent constant analysis of structure–activity relationships.

Experimental

The partition coefficients were determined according to our previously reported[4] procedure. Most of the compounds whose log P values are reported in Tables I and V were purified for partitioning by preparative vapor phase chromatography. Several of the compounds employed in this work have not been reported previously.

1-Fluoro-3-phenylpropane.—A mixture of 1-chloro-3-phenylpropane (35 g.), dry powdered potassium fluoride (21 g.), and 120 ml. of ethylene glycol was heated at 150–160° for 12 hr. with vigorous stirring. The mixture was then cooled, diluted with water, and extracted with ether. Evaporation of the ether and fractionation of the residue yielded 11 g. of product boiling from 173–195°. This material was purified for partitioning by means of an Aerograph autoprep using a silicon column; b.p. 183.5° (730.5 mm.), $n^{23}D$ 1.4870.

Anal. Calcd. for $C_9H_{11}F$: C, 78.26; H, 7.97. Found: C, 78.01; H, 8.12.

2-Amino-4-phenylpentanoic acid was prepared by the malonic ester method.[11] The melting point of our product after recrystallization from water was 245–246° dec.; von Braun and Kruber[11] reported 203–206°.

Anal. Calcd. for $C_{11}H_{15}NO_2$: C, 68.42; H, 7.76. Found: C, 68.47; H, 7.83.

4-Cyclopropyl-2-butanone.—Cyclopropylmethyl bromide was condensed with ethyl acetoacetate in the usual way.[12] The resulting product was hydrolyzed with 5% KOH (yield 33%). The crude material was purified by vapor phase chromatography; b.p. 155° (732 mm.), $n^{25}D$ 1.4260.

Anal. Calcd. for $C_7H_{12}O$: C, 74.94; H, 10.78. Found: C, 74.89; H, 10.74.

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The Formation of O-Methylated Catechols by Microsomal Hydroxylation of Phenols and Subsequent Enzymatic Catechol O-Methylation. Substrate Specificity

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A microsomal hydroxylating system which converts phenols to catechols and requires niacinamide adenine dinucleotide phosphate and glucose-6-phosphate has been assayed for a variety of phenols using the enzyme catechol-O-methyltransferase and radioactive S-adenosylmethionine-methyl-C^{14}. This system specifically methylates catechols converting them to radioactive methoxyphenols which can be extracted and assayed. Among the phenols which are converted to catechols are N-acetylserotonin, hydroxyindoles, tyramine, octopamine, hordenine, metanephrine, morphine, phenazocine, levorphanol, and estradiol. 2,4,6-Trichlorophenol formed an O-methylated product. Products from a variety of substrates were identified by cochromatography with authentic compounds.

Liver microsomes have been shown to hydroxylate a variety of aromatic compounds to phenols.[1–3] Recently, Axelrod[4] has demonstrated that phenolic amines are further hydroxylated by microsomal preparations to yield catechol amines such as (nor)epinephrine and dopamine. The conversion of tyramine to norepinephrine has been demonstrated *in vivo*,[5] a finding that focuses attention on the group of microsomal hydroxylases which convert phenols to catechols. Among the compounds containing phenolic groups and thus potential substrates for the formation of catechols are various physiologically active hydroxyindoles and phenolic phenethylamines, and a large number of important drugs (morphine, levorphanol, etc.). In order to carry out a survey of substrates, a convenient and widely applicable assay was needed. Catechol-O-methyltransferase[6] is an enzyme occurring in the soluble supernatant fraction of homogenized liver, which readily

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